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Blood and Sputum Cytology in Patients of Bronchial Asthma and their Correlation with Fractional Exhaled Nitric Oxide

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Abstract

Introduction: Asthma is a heterogeneous chronic airway disease characterised by variable respiratory symptoms and airflow limitation. Recent evidence suggests that asthma consists of distinct inflammatory phenotypes identified through sputum cytology and supported by biomarkers such as peripheral blood eosinophils and Fractional Exhaled Nitric Oxide (FeNO).

Aim: To evaluate the correlation between blood and sputum differential leukocyte counts and FeNO levels in patients with bronchial asthma.

Material and Methods: This analytical cross-sectional study included previously or newly diagnosed asthma patients aged 18 - 60 years attending the Department of Respiratory Medicine from April 2024 to November 2025. A total of 152 participants were enrolled using systematic sampling (K = 3). All subjects underwent clinical assessment, spirometry, blood differential leukocyte count, induced sputum cytology, chest radiography, and FeNO measurement. Data were analyzed using SPSS version 22. Correlations were assessed using Karl Pearson's co-efficient, and associations were tested with Chi-square or Fisher's exact test.

Results: Most participants were aged 30 - 44 years, with eosinophilic asthma being the most common phenotype. Blood eosinophilia (p = 0.001), absolute eosinophil count (p = 0.000), and sputum eosinophils (p = 0.000) showed significant associations with FeNO levels. A weak positive correlation was observed between blood and sputum eosinophils (r = 0.256, p = 0.001), while FeNO showed a moderate positive correlation with blood eosinophils (r = 0.406, p = 0.000). Higher FeNO values were observed in eosinophilic asthma phenotypes.

Conclusion: Blood and sputum eosinophil counts correlate with FeNO levels and may serve as reliable non-invasive markers of eosinophilic airway inflammation, supporting phenotype-based asthma assessment for personalised management.

Key words: Asthma, FeNO, blood eosinophils, sputum cytology, airway inflammation, biomarkers.

Introduction

Asthma is a heterogeneous disease usually characterised by chronic airway inflammation that affects approxmately 358.2 million people worldwide[1]. It is defined by a history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity together with variable expiratory airflow limitation. Thus, causing a substantial public health problem[2]. In India, it affects approximately 30 million people (0.96 - 11.03%)[3]. It has been recognised that among asthmatic patients there are clusters of demographics, clinical and/or pathophysiological characteristics, which have been called "asthma phenotypes"[4,5].

The pathogenetic pathway of asthma is the Th2 driven pathway which recruits eosinophil in the airway, but there is also a varied pathogenesis occurring in the airway of chronic persistent form of asthma inviting neutrophils and other granulocytes in the airway[6]. However, to date, no strong relationship has been found between specific pathological features and particular clinical or treatment responses[7].

When examining the airway inflammation using sputum analysis, patients with asthma can be classified into four different inflammatory phenotypes. Based on the percentage of eosinophils and neutrophils in sputum, one can define four airway inflammatory subgroups: eosinophilic asthma, neutrophilic asthma, mixed granulocytic asthma and paucigranulocytic asthma[8]. There is recent evidence from prospective clinical studies that airway infammatory phenotype can help to optimise therapy and disease outcome[9].

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Airway inflammatory phenotype can be measured through the airway noninvasively by induced sputum analysis[4,8] and fractional exhaled nitric oxide (FeNO)[10]. Both are considered direct, reliable, sensitive, simple, and repeatable methods of assessing inflammatory phenotypes, widely used in clinical practice.

To assess the phenotype, sputum cytology is a simple non-invasive test. Understanding the type of exacerbation and population of cells in sputum cytology may guide in the treatment of bronchial asthma. Increased sputum neutrophil count was found to be associated with enhanced chemotactic activity of peripheral blood neutrophils during allergen-induced late-phase airway inflammation in patients with allergic asthma.

Asthmatic patients with a higher FeNO level had a higher blood eosinophil count, and lower FEV1 values. Peripheral blood eosinophils has also been studied as another potential biomarker; it is associated with the patient's future risk for exacerbations[11,12], for developing fixed airflow limitation[13], and a diagnosis of eosinophilic asthma[14].

Limited studies have correlated blood and sputum cytology with FeNO[9]. The results of the present study will aid present to predict airway eosinophilic/neutrophilic inflammation and provide guidance for the individualised clinical treatment of patients with asthma.

We conducted a hospital based cross-sectional study and the aims of our study were (1) To estimate sputum and blood differential leucocyte count in Bronchial Asthma, (2) To estimate level of FeNO, (3) To study the relation of sputum cytology and blood cytology with FeNO.

Methods

Study Design and participants

We conducted a study on a series of 152 patients with asthma visiting our hospital between April 2024 and November 2025. Inclusion criteria were patients of age 18 - 60 years. Previously as well as newly diagnosed cases of asthma. Patient who give consent to be part of study.

Exclusion criteria were: Smokers (A patient who has smoked more than 100 cigarettes/bidi in his life) patients of chronic obstructive pulmonary disease as well as ACOS patients with active as well as healed pulmonary tuberculosis. Cases were enrolled only after ethical clearance.

All recruited patients (previously as well as newly diagnosed of asthma as per GINA guidelines 15 underwent: Detailed clinical history including past history: similar illness, previous hospital admissions, steroid use, exposure to allergens. Chest x-ray PA view was done to rule-out active pulmonary diseases. Anthropometric measurements were done. Spirometry with bronchodilator was done for every patient to confirm the diagnosis of asthma. Dyspnoea was graded as per Modified Medical Research Council (mMRC) Grading[16]. Severity of symptoms were adjusted according to: Asthma control questionnaire[17]. Blood differential leucocyte count-Neutrophils, Eosinophils, Monocytes and Lymphocytes was sent. Sputum for routine microscopy including – Neutrophils, Eosinophils, Lymphocytes and macrophages was sent. FeNO (Fractional Exhaled Nitric Oxide) measurements was done.

Patients were then divided into different groups on the basis of diferential counts of sputum and blood eosinophils. Patients with sputum eosinophils > = 2% were labelled as sputum eosinophilia group and patients with sputum eosinophils <2% were labelled as sputum non-eosinophilia group[18]. Patients with blood eosinophils >6% were labelled as blood eosinophilia group and patients with blood eosinophils < = 6% were labelled as blood non-eosinophilia group.[18]Then these four groups were compared with various clinical parameters.

Age was distributed among 3 groups : less than 30 yrs, 30 - 44 years and 45 - 60 years. BMI categorisation was done according to WHO categorisation[19]. Socio-economic status was divided according to latest Kuppuswamy socio-economic scale[20]. Dyspnoea categorisation was done according to mMRC grading[16]. Absolute eosinophils were divided into two groups <150 and >= 150[21]. Pre-FEV_1 was divided into 7 groups[17]. Severity of asthma was categorised on the basis of asthma control questionnaire[17], and FeNO was divided into two groups of <= 25 ppb and >25ppb[21].

Statistical Analysis

Statistical testing was conducted with SPSS (version 22). Continuous variables were presented as mean ± SD and median for normally distributed data. Categorical variables were expressed as frequencies and percentages. Correlation between two quantitative variables was tested by Karl Pearson's co-efficient of correlation. To test the association between 2 categorical variables we used Chi-squared test or Fisher's exact test. A p-value of less than or equal to 0.05 was considered as statistically significant.

Results

In this study, population had a mean age of 35.57 ± 12.05 years (range 18 - 60), with most participants aged 30 - 44 years (38.8%), followed by those under 30 (36.2%) and 45 - 60 years (25%), indicating bronchial asthma

predominantly affected younger and middle-aged adults. Gender distribution was nearly equal (females 50.7%, males 49.3%), minimizing sex-based confounding. Anthropometric assessment showed a mean height of 159.65 cm, weight 61.43 kg, and BMI 24.12 kg/m^2, with the majority having normal BMI (55.9%), while 30.3% were overweight, 7.9% obese, and 5.9% underweight (Table I).

Sputum Eosinophils

The correlation of sputum eosinophilia with demographic and clinical parameters indicates that younger patients tended to have higher rates of sputum eosinophilia, with 63.6% of those under 30 years showing ≥2% eosinophils, compared with 54.2% in the 30 - 44 age group and 39.5% in the 45 - 60 group, although this trend did not reach statistical significance (p = 0.071). Dyspnoea severity (mMRC) showed a tendency for higher sputum eosinophilia in patients with more severe breathlessness, particularly grade 3 (87.5%) and grade 2 (58.9%), compared with grade 1 (47.7%), though the relationship narrowly missed significance (p = 0.062). Overall, sputum eosinophilia appeared more common in younger and more symptomatic patients, but demographic factors [Gender (p = 0.616), socio-economic status (p = 0.454)] and BMI (p = 0.353) did not significantly influence airway eosinophilic inflammation. The analysis of sputum eosinophilia in relation to clinical and laboratory parameters showed a strong association with blood eosinophilia, as 74.4% of patients with blood eosinophilia also had sputum eosinophilia, compared with 45.9% among those with blood non-eosinophilia (p = 0.001). Similarly, absolute blood eosinophil count was significantly correlated, with 63.6% of individuals having blood AEC ≥150 showing sputum eosinophilia, whereas only 31.1% of those with blood AEC <150 did so (p = 0.000). Pre-FEV1 percentages did not demonstrate a statistically significant relationship (p = 0.323). Asthma control as assessed by ACQ showed no significant correlation with sputum eosinophilia (p = 0.483), with elevated eosinophils present across all levels of control. In contrast, FeNO levels were strongly associated with sputum eosinophilia; 70.8% of patients with FeNO >25 ppb exhibited sputum eosinophilia, compared with 38.8% with FeNO ≤25 ppb (p = 0.000), highlighting FeNO as a robust marker of airway eosinophilic inflammation (Tables II and III).

Peripheral Blood Eosinophils

Across the study population, blood eosinophilia (>6%) showed a significant association with age, with higher eosinophil levels observed more commonly in participants younger than 30 years (40%) compared to those aged 30 - 44 years (25.4%) and 45 - 60 years (15.8%), as indicated by a p-value of 0.032. No significant gender difference was noted, as eosinophilia was nearly identical between males (28%) and females (28.6%) with a non-significant p-value of 0.938. BMI categories also showed no meaningful association with eosinophilia (p = 0.277). Socio-economic status did not demonstrate a significant relationship with eosinophil levels (p = 0.158). Dyspnoea severity (mMRC) likewise exhibited no significant association (p = 0.099). Sputum eosinophilia ≥2% was significantly linked to higher blood eosinophil levels (39%) compared with sputum eosinophilia <2% (15.7%), with a highly significant p-value of 0.001. Absolute eosinophil count also demonstrated a marked relationship, as only 2.2% of individuals with counts <150 had blood eosinophilia, whereas 39.3% of those with counts ≥150 showed elevated blood eosinophils (p = 0.000). Pre-FEV1 values did not show statistically significant differences (p = 0.138). Asthma control showed a significant correlation, where poorly controlled asthma (ACQ >1.51) had 29.4% eosinophilia, while none of the well-controlled patients (ACQ <0.75) exhibited eosinophilia (p = 0.010). Fractional exhaled nitric oxide (FENO) was also significantly associated, with eosinophilia being more prevalent among individuals with elevated FENO >25 ppb (41.7%) compared with those having FENO ≤25 ppb (16.3%), supported by a p-value of 0.001 (Tables II and III).

Table I: Mean of various parameters

Parameter	Values
Age (Mean ± SD)	35.57 ± 12.05 yrs
Males n (%)	75 (49.3%)
Females n (%)	77 (50.7%)
Height (Cms) (Mean)	159.65
Weight (Kgs) (Mean)	61.428
BMI (kg/m^2) (Mean)	24.117
Pre-FEV_1 (%) (Mean)	57.2%
Ratio FEV_1/FVC (%) (Mean)	59.7%.

Table II: Correlation of sputum and blood eosinophils with various parameters

Parameters	Sputum Eosinophilia(>=2%)	Sputum Non-eosinophilia (<2%)	p-value	Blood Eosinophilia (>6%)	Blood Non-eosinophlia (<6%)	p- value
Age (years)						
<30	35 (63.6%)	20 (36.4%)	0.071	22(40%)	33(60%)	**0.032**
30 - 44	32 (54.2%)	27 (45.8%)		15(25.4%)	44(74.6%)	
45 - 60	15 (39.5%)	23 (60.5%)		6(15.8%)	32(84.2%)	
Gender						
Male	42 (56.0%)	33 (44.0%)	0.616	21 (28%)	54 (72%)	0.938
Female	40 (51.9%)	37 (48.1%)		22 (28.6%)	55 (71.4%)	
BMI (kg/m^2)						
<18.5	5 (55.6%)	4 (44.4%)	0.353	3 (33.3%)	6 (66.7%)	0.277
18.5 - 24.9	51 (60.0%)	34 (40.0%)		28 (32.9%)	57 (67.1%)	
25 - 29.9	21 (45.7%)	25 (54.3%)		8 (17.4%)	38 (82.6%)	
>30	5 (41.7%)	7 (58.3%)		4 (33.3%)	8 (66.7%)	
Socio-economic Status						
Lower Middle	29 (48.3%)	31 (51.7%)	0.454	22 (36.7%)	38 (63.3%)	0.158
Upper Lower	47 (58.8%)	33 (41.3%)		19 (23.8%)	61 (76.3%)	
Upper Middle	6 (50.0%)	6 (50.0%)		2 (16.7%)	10 (83.3%)	
Dyspnoea grade (as per mMRC)						
3	7 (87.5%)	1 (12.5%)	0.062	3 (37.5%)	5 (62.5%)	0.099
2	33 (58.9%)	23 (41.1%)		21 (37.5%)	35 (62.5%)	
1	42 (47.7%)	46 (52.3%)		19 (21.6%)	69 (78.4%)	

Table III: Correlation of sputum and blood eosinophils with various parameters

Parameters	Sputum Eosinophilia (>=2%)	Sputum Non-eosinophilia (<2%)	p-value	Blood Eosinophilia (>6%)	Blood Non-eosinophlia (<6%)	p- value
Sputum Eosinophilia						
<2%	-	-		11 (15.7%)	59 (84.3%)	**0.001**
>=2%	-	-		32 (39.0%)	50 (61.0%)	
Blood Eosinophilia						
<=6%	50 (45.9%)	59 (54.1%)	0.001	-	-	-
>6%	32 (74.4%)	11 (25.6%)		-	-	
Absolute Eosinophil Count in Blood						
<150	14 (31.1%)	31 (68.9%)	0.000	1 (2.2%)	44 (97.8%)	**0.000**
>=150	68 (63.6%)	39 (36.4%)		42 (39.3%)	65 (60.7%)	
Pre FEV1 %						
<50%	30 (48.4%)	32 (51.6%)	0.323	21 (33.9%)	41 (66.1%)	0.138
50% - 59%	19 (67.9%)	9 (32.1%)		5 (17.9%)	23 (82.1%)	
60% - 69%	15 (62.5%)	9 (37.5%)		10 (41.7%)	14 (58.3%)	
70% - 79%	6 (40.0%)	9 (60.0%)		3 (20%)	12 (80%)	
80% - 89%	7 (50.0%)	7 (50.0%)		1 (7.1%)	13 (92.9%)	
90% - 95%	2 (100%)	0 (00.0%)		0 (0.0%)	2 (100%)	

>95%	3 (42.9%)	4 (57.1%)		3 (42.9%)	4 (57.1%)	
Asthma Control Questionnaire						
<0.75	5 (41.7%)	7 (58.3%)	0.483	0 (00.0%)	12 (100%)	**0.010**
0.75-1.5	3 (75.0%)	1 (25.0%)		3 (75.0%)	1 (25.0%)	
>1.51	74 (54.4%)	62(45.6%)		40 (29.4%)	96 (70.6%)	
FeNO						
<=25 ppb	31 (38.8%)	49 (61.3%)	**0.000**	13(16.3%)	67 (83.8%)	**0.001**
>25 ppb	51 (70.8%)	21 (29.2%)		30 (41.7%)	42 (58.3%)	

Discussion

The present study evaluated the association between inflammatory biomarkers – blood eosinophils, sputum eosinophils, and fractional exhaled nitric oxide (FeNO) – in patients with bronchial asthma. Understanding the relationship between these biomarkers is important for identifying airway inflammation and for tailoring personalised treatment strategies. Our findings highlight the potential role of non-invasive biomarkers in guiding asthma management and predicting treatment response.

The present study, suggests that bronchial asthma predominantly affects younger and middle-aged adults in our cohort. Anthropometric assessment revealed that the majority of patients had BMI within or slightly above the normal range, which may influence asthma severity and inflammatory profiles. These demographic findings are comparable to previous studies. Ganapathy *et al* reported a mean age of 41 years, with the majority of participants in the 35 - 45 year age group, and a gender distribution of 49% males and 51% females[22]. Similarly, Lobiyal *et al* reported a mean age of 30 years among patients with bronchial asthma[23]. Compared with these studies, the mean age in our cohort was slightly lower (36 years), while the gender distribution remained nearly equal.

Baseline spirometry in our study demonstrated moderate airflow limitation, with mean pre-bronchodilator FEV_1 of 57.2% predicted and mean FEV_1/FVC ratio of 59.7% (Table I). Additionally, 89.5% of participants had poor asthma control according to Asthma Control Questionnaire (ACQ) scores. In contrast, Gao *et al* reported a mean FEV_1% predicted value of 81.77% and a mean FEV_1/FVC ratio of 68.33%, suggesting relatively milder airflow limitation in their study population[18]. Similarly, Nakwan *et al* observed a mean FEV_1% predicted value of 77.6% with an interquartile range of FEV_1/FVC ratio between 0.63 and 0.78[24]. These findings suggest that airflow limitation in our cohort was comparatively more pronounced. Furthermore, pulmonary

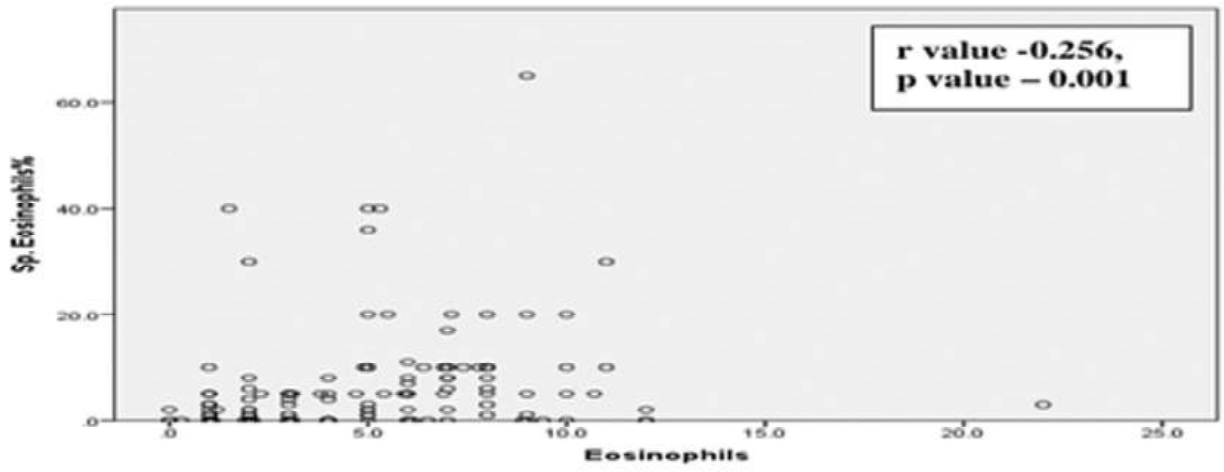

Fig. 1: *Correlation between blood eosinophils and sputum eosinophils.*

Scatter plot showing the relationship between sputum eosinophil percentage on y-axis and blood eosinophil percentage on n-axis. The r-value (0.256) indicates a weak positive correlation between blood eosinophils and sputum eosinophils. The p-value (0.001) is statistically significant. Overall, the figure demonstrates that although sputum and blood eosinophil levels are weakly correlated, the association is statistically meaningful, implying that blood eosinophil percentage may provide some – but limited – predictive information about sputum eosinophilic inflammation.

function parameters in our study did not show significant correlations with sputum or blood eosinophil levels.

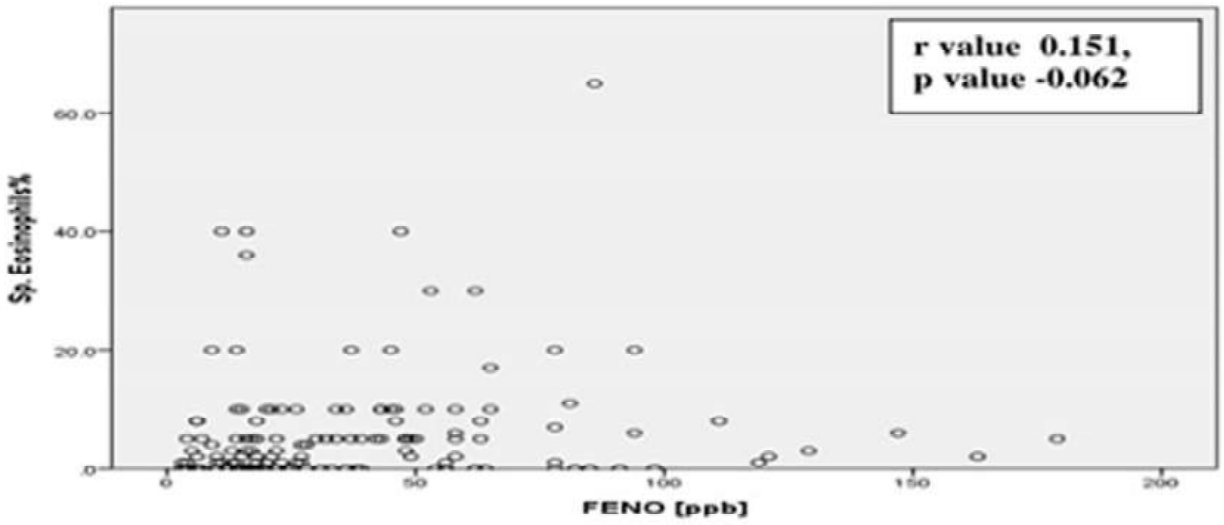

Fig. 2: *Correlation between sputum eosinophils and FeNO.*

Scatter plot showing the relationship between sputum eosinophil percentage on y-axis and fractional exhaled nitric oxide (FeNO) levels on x-axis. The r-value (0.151) indicates a very weak positive correlation between FeNO and sputum eosinophils. The p-value (0.062) is not statistically significant. Overall, the figure demonstrates that although there is a small positive trend, FeNO does not significantly correlate with sputum eosinophilic inflammation in this sample.

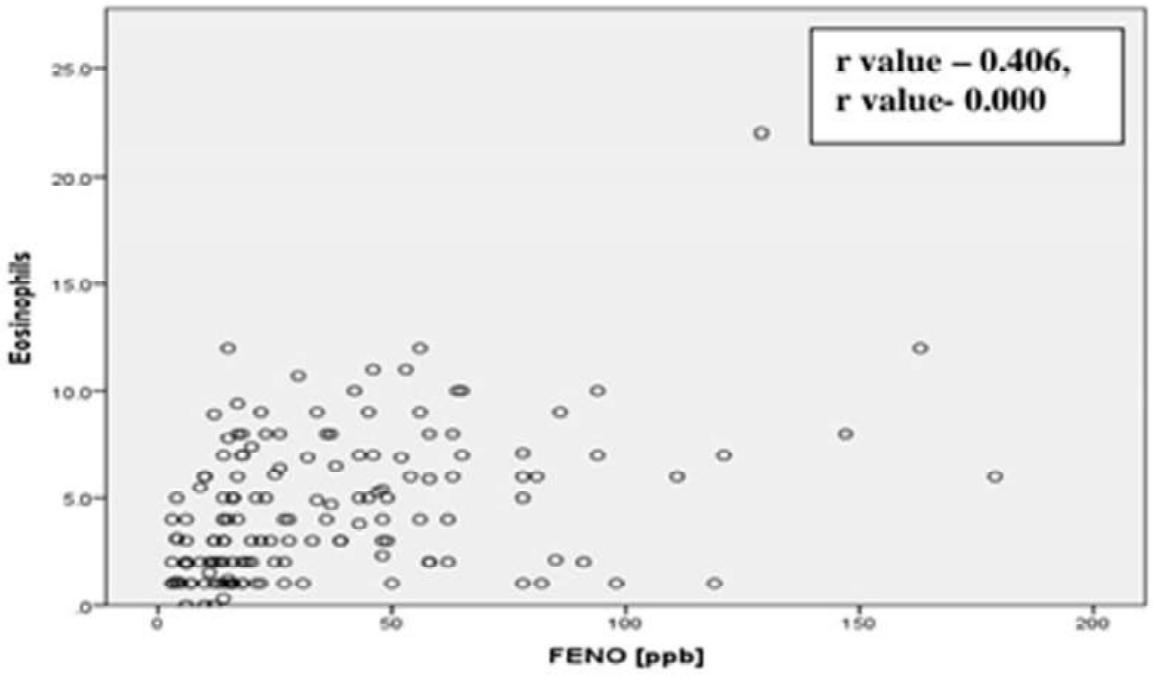

Fig. 3: *Correlation between blood eosinophils and FeNO.*

Scatter plot showing the relationship between blood eosinophil percentage on y-axis and FeNO (fractional exhaled nitric oxide) levels on x-axis. The r-value (0.406) indicates a moderate positive correlation between blood eosinophils and FeNO. The p-value (0.000) is highly statistically significant (p <0.001). Overall, the figure demonstrates a statistically significant and moderately strong relationship between systemic eosinophilia and exhaled nitric oxide, supporting FeNO as an important non-invasive marker of eosinophilic inflammation.

With respect to inflammatory biomarkers, blood eosinophilia (>6%) was observed in 28.3% of participants, while 70.4% had absolute eosinophil counts ≥150 cells/μL. Sputum analysis demonstrated a median neutrophil proportion of 57.5% and sputum eosinophils of 2%. Elevated FeNO levels (>25 ppb) were detected in 47.4% of patients. Importantly, FeNO levels showed a significant positive correlation with blood eosinophilia (r = 0.406, p <0.001) (Fig. 3), whereas the correlation with sputum eosinophils was weak and not statistically significant (r = 0.151, p = 0.062) (Fig. 2). These findings suggest that blood eosinophil counts may serve as a more reliable indicator of eosinophilic airway inflammation in this cohort.

Correlation analysis demonstrated moderate positive associations between sputum eosinophils and both blood eosinophil counts (r = 0.346, p <0.001) (Fig. 4) and eosinophil percentages (r = 0.256, p = 0.001) (Fig. 1). Additionally, blood eosinophil counts were strongly correlated with FeNO levels (r = 0.406, p <0.001) (Fig. 3). Conversely, sputum neutrophils exhibited weak negative correlations with blood eosinophil percentages and absolute eosinophil counts. These observations indicate the coexistence of different inflammatory phenotypes in asthma, including eosinophilic and neutrophilic patterns.

Our findings are broadly consistent with previous studies. Shi *et al* reported a positive correlation between ACQ scores and sputum eosinophil counts, while ACT scores were negatively correlated with sputum eosinophilia. Furthermore, blood eosinophil counts and proportions were significantly correlated with sputum eosinophil proportions, and FeNO levels showed a positive correlation with sputum eosinophilia[25]. Similarly, Marineau *et al* observed low-to-moderate positive correlations between sputum eosinophil percentage and FeNO levels, as well as between sputum eosinophils and blood eosinophils[26]. Gao *et al* also reported significant positive correlations between FeNO levels and both sputum eosinophil percentages and blood eosinophil counts, while demonstrating a negative correlation with sputum neutrophils[18]. Nakwan *et al* reported a median FeNO level of 23 ppb and blood eosinophil count of 375 cells/mm^3, with a significant positive correlation between FeNO and blood eosinophils (r = 0.310, p = 0.004)[24]. Taken together, these findings indicate a consistent association between FeNO levels, blood eosinophils, and sputum eosinophils, reflecting the link between systemic eosinophilia and airway eosinophilic inflammation. However, the strength of these associations varies across studies, highlighting heterogeneity in asthma inflammatory phenotypes. Additionally, FeNO appears to be a poor surrogate marker for sputum neutrophilic inflammation.

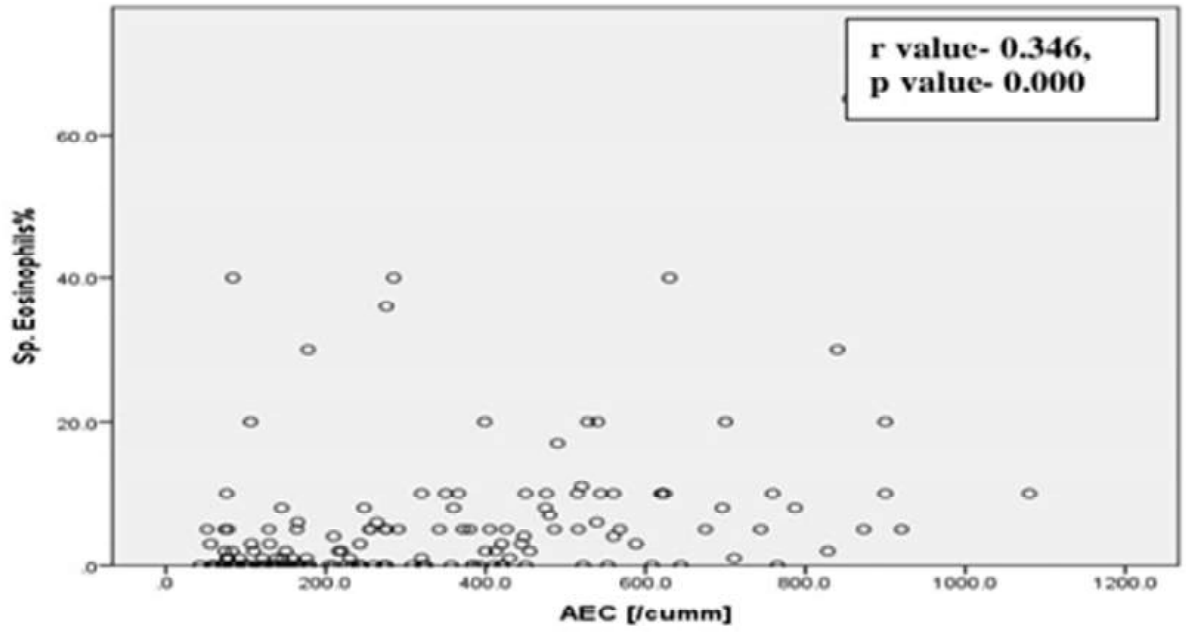

Fig. 4: *Correlation between sputum eosinophils and absolute eosinophil count.*

Scatter plot showing the relationship between sputum eosinophil percentage on y-axis and absolute eosinophil count (AEC) in peripheral blood on x-axis. The r-value (0.346) indicates a moderate positive correlation between AEC and sputum eosinophils. The p-value (0.000) is highly statistically significant (p <0.001). Overall, the figure demonstrates a significant and moderately strong relationship between peripheral blood eosinophil count and sputum eosinophilic inflammation, implying that AEC may serve as a useful indicator of airway eosinophilia.

Overall, the present study reinforces the importance of integrating biomarker assessment into routine asthma evaluation. The combined assessment of FeNO and blood eosinophil counts may provide valuable insight into airway inflammatory patterns and support personalised treatment strategies.

Conclusion

The study demonstrates a significant association between eosinophilic inflammation and FeNO levels in patients with bronchial asthma. Blood and sputum eosinophil counts reliably reflect airway eosinophilia and correlate with FeNO, supporting their role as accessible biomarkers for asthma. Therefore a holistic approach for clinical evaluation and biomarker assessment should guide personalised management rather than relying solely on patient characteristics. Future studies with larger sample sizes and longitudinal follow-up are required to validate these findings and to further elucidate the dynamic changes in asthma phenotypes over time.

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